Otolith shape analysis of three gobiid species of the Northwestern Black Sea and characterization of local populations of *Neogobius melanostomus*

by

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Abstract. – The present study describes the otolith shape and age of three species of Gobiidae: *Neogobius melanostomus* (Pallas, 1814), *Ponticola eurycephalus* (Kessler, 1974) and *Mesogobius batrachocephalus* (Pallas, 1814) from the Western part of the Black Sea. These phylogenetically close species showed otolith shape differences. The round goby, *Neogobius melanostomus*, showed differences in otolith shape between local populations, probably related to differences in diet and environmental variability influenced by the Danube River inputs in the North of the study area. The age of these commercially exploited species was estimated by otolith microstructural analysis and the results were linked to sex, size and weight. This information is valuable for fishery management purposes.

Résumé. – Analyse de la forme des otolithes de trois espèces de Gobiidae du nord-ouest de la mer Noire et caractérisation des populations locales de *Neogobius melanostomus*.

Cette étude décrit la forme des otolithes et l'âge de trois espèces de Gobiidae de la partie nord-ouest de la mer Noire : *Neogobius melanostomus* (Pallas, 1814), *Ponticola eurycephalus* (Kessler, 1974) et *Mesogobius batrachocephalus* (Pallas, 1814). Ces espèces, proches phylogénétiquement, présentent des différences dans la forme de leurs otolithes. De plus, chez le gobie à taches noires, *Neogobius melanostomus,* la forme des otolithes est différente entre les populations locales. Cela est probablement lié aux différences d'alimentation et à la variabilité environnementale, influencée par les apports du Danube dans la partie nord de la zone d'étude. Pour ces espèces exploitées et commercialisées, les âges estimés ont été reliés au sexe et aux mesures biométriques pour la gestion des pêcheries.

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Gobies are a Ponto-Caspian relict and Romanian species are well adapted to the variability of environmental parameters (Mihălcescu, 2005). In the Northwestern Black Sea, the Romanian coast is characterized by a high variability of physical, chemical and biological parameters (Nicolaev and Zaharia, 2011), in particular related to the Danube River inputs in the northern area (Bănaru *et al.,* 2007). The northern area is mainly sandy, while in the South sandy zones are discontinuous and generally represent small "islands" between rocky zones (Bănaru *et al*., 2007).

The most abundant goby species in the Romanian marine area are the round goby *Neogobius melanostomus* (Pallas, 1814) (= *Apollonia melanostomus,* Stepien and Tumeo, 2006), the mushroom goby *Ponticola eurycephalus* (Kessler, 1974) and the knout goby *Mesogobius batrachocephalus* (Pallas, 1814) (Neilson and Stepien, 2009). These three species included in the "Gobius-lineage" are phylogenetically close (Thacker and Roje, 2011; Agorreta *et al*., 2013). The round goby is able to tolerate high salinity variations, being present in both northern and southern parts of the Romanian coast and showing important differences in diet between these areas (Bănaru *et al*., 2007). The mushroom goby and the knout goby are located only in the southern area. The three species have no migratory behaviour. Eggs and larvae of the three species are benthic with reduced mobility for the round goby and the mushroom goby and slightly higher for the knout goby. Gobies play an important role in benthic food web, being bivalves, small teleosts and other small prey predators, and being themselves consumed by turbot (Bănaru and Harmelin-Vivien, 2009).

Otoliths, calcium carbonate structures located in the fish inner ear, have a role in audition and balance mechanisms. They are an indirect means to study fish populations and assess the relationship between the environment and the

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organisms (Panfili *et al.,* 2002). Otolith shape has long been known to be species specific (L'Abée-Lund, 1988). Despite their phylogenetic proximity, these goby species may show interspecific differences in otolith shape. Otolith shape appears to be an ideal marker for teleost populations (Campana and Casselman, 1993; Tracey *et al.,* 2006; Morat *et al.,* 2008, 2012); indeed otolith shape analysis can show diet and environmental differences by intraspecific differences in their shape. Otolith shape analyses of Gobiidae have previously been performed in other areas (Lord *et al.,* 2012), but not in the Mediterranean and the Black Sea.

Otoliths exhibit incremental structures formed regularly over time, which can be used for age estimation (Panfili *et al.,* 2002). In addition, otoliths are considered to be a "flight recorder" of the life of a fish (Lecomte-Finiger, 1999). They grow throughout the fish life and are metabolically inert structures; once deposited, otolith material is unlikely to be resorbed or altered (Campana and Neilson, 1985). Age estimations of Gobiidae species have previously been achieved in other areas (Kritzer, 2002), in the Mediterranean (Iglesias *et al.,* 1997) and in the southern part of the Black Sea (Gümüş and Kurt, 2009).

Otolith shape analyses and age estimations may be particularly useful for fisheries management purposes in order to discriminate spatial stocks (Xin *et al*., 2014) and to relate age, sex and size for population analysis. They may provide valuable information regarding the age structure of an exploited population. At present, fishing in the study area is mainly artisanal (small scale fisheries), and the Romanian fishing fleet has declined strongly over the last decades because of the decline of stocks and the disappearance of many species (Bănaru *et al.,* 2010, 2015; Creţeanu, NAFA (National Agency for Fisheries and Aquaculture Romania), pers. comm.). While the other species have become increasingly rare, the percentage of gobies in total landings has increased over the last decades (FAO, 2005; Creţeanu, NAFA (National Agency for Fisheries and Aquaculture Romania), Pers. Comm.). Between 2006 and 2010, gobies occupied the 5th place in terms of landed biomass in this area. *Neogobius melanostomus* dominates these catches (97%), *Ponticola eurycephalus* represents almost 3% of the catches while the other species represented less than 1%. In 2010, there were 215 fishermen and 89 boats fishing for gobies (FAO, 2005; Creţeanu, NAFA (National Agency for Fisheries and Aquaculture Romania), Pers. Comm.). They constitute the basis of small artisanal fisheries and a means of subsistence for local fishermen (Fig. 1). Despite their importance, there are relatively few data on their ecology and no studies concerning their otoliths. This is the first paper to focus on the otolith shape of gobies in the Black Sea and the first concerning their age in the western part of the Black Sea.

The main aims of this paper were to (i) show interspecific differences in otolith shape of the three species studied, (ii) test whether the environmental variations may induce differences in otolith shape of the round goby, and (iii) give age estimations of gobies sampled from commercial catches related with sex and biometric measures, which may be useful for fishery management.

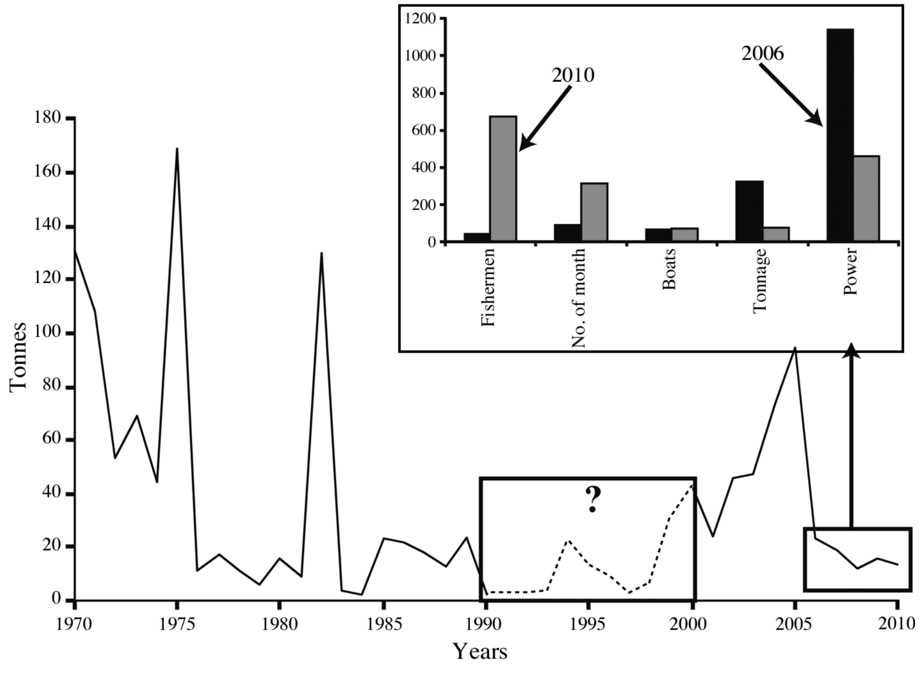

Figure 1. – Landings of gobies on the Romanian Black Sea coast between 1970 and 2010. Data sources: FAO 2005; Creţeanu, NAFA (National Agency for Fisheries and Aquaculture Romania, pers. comm.). Uncertainty in data sources between 1989 and 2000 are indicated by dotted line. Fishing effort in terms of number of fishermen, cumulated number of months of activity for all fishermen, number of boats, tonnage and power of engines are indicated for the last period (2006 and 2010).

MATERIALS AND METHODS

Study regions

The study area is located in the Northwestern part of the Black Sea coast, seawards of the Danube River delta (45°12’-43°44’N, 29°40’-28°35’E) (Fig. 2). The Romanian coastline, 244 km long, can be divided into two main geographical and geomorphological areas. The northern area (165 km in length) is low, accumulative, mainly sandy adjacent to the Danube Delta, which represents the main source of sediment for the littoral system. The southern area (about 75 km in length) is characterized by erosion, with active cliffs and small beach barriers separating coastal lagoons from the sea (Panin, 2005). These two areas have different hydrological, physical, chemical, sedimentary and biological characteristics. Sandy zones are widespread (about 700 km^2) all along the Romanian Black Sea shore. Compared to sandy bottoms, the rocky natural bottoms occupy a much smaller surface area (0.3% of the total surface area of the Romanian coast), and are located only in the south. Here, the sandy zones are discontinuous and generally represent small “islands” between stony zones (Postolache, 1990). In all areas, sediments become finer as depth increases, and sand is replaced by silt (Petranu, 1997). Mud dominates in deeper zones.

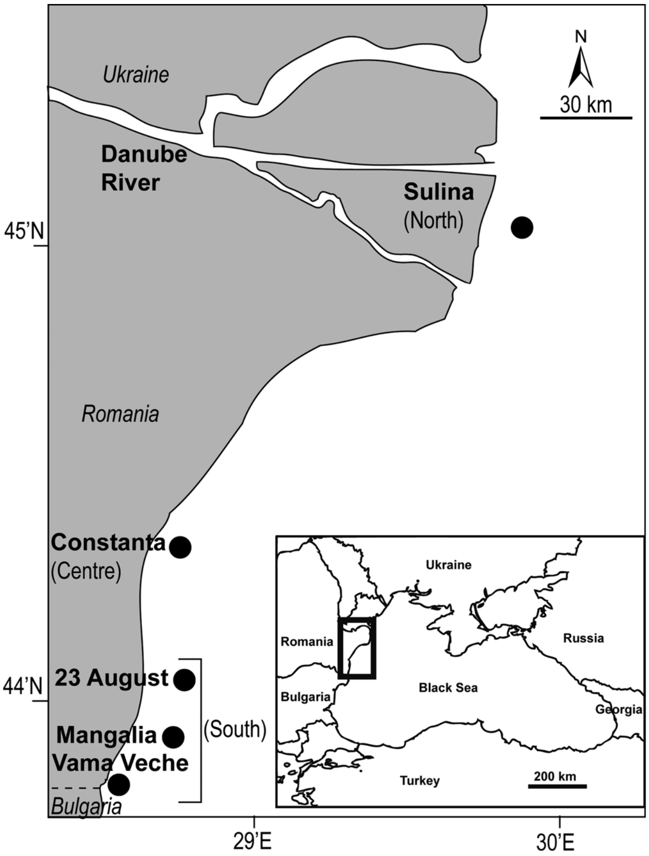

Figure 2. – Map of the sampling stations in the Northwestern Black Sea along the Romanian coast.

Coastal waters are influenced by the Danube inputs, which supply large amounts of organic and inorganic discharges. Mean annual Danube River flow is 6,500 m^3 s^{-1}. Direction, range and depth of the expansion of Danube River water masses are variable and depend on water discharge intensity and wind direction. The general cyclonic current in the Black Sea flows from north to south along the Romanian coast. It is strengthened by the north winds and is responsible for the occurrence of particular, rather estuarine conditions in the western part of the Pontic Basin (Petranu, 1997). Under Danube River and wind influence, seawaters along the Romanian coast are characterized by strong spatial and seasonal changes in temperature (0-27°C) and salinity (4-17) (Berlinsky *et al.,* 2006). Mean salinity in the Black Sea is about 17-18 in surface waters and 22 in deep waters.

Sampling

The three species were sampled in the Northwestern Black Sea between 10 and 50 m deep along the Romanian coast by lines and pound nets in 2005 and 2006. Individuals were collected in Sulina in the North (off the Danube River mouth), Constanta in the centre (near the harbour city of Constanta), 23 Aug., Mangalia and Vama Veche in the South (Fig. 2; Tab. I).

Total individual length (TL cm) and mass (g) were recorded for both male and female individuals. The sex of the individuals was noted based on their gonad development. Sagittal otoliths were extracted for analyses, cleaned with distilled water and stored in dry tubes.

Otolith shape analyses

Each otolith, systematically placed with the *sulcus acusticus* oriented towards the observer, was examined under a stereo-microscope (Leica MZ16) fitted with a digital camera (Media Cybernetics evolution LS color, Inc. Bethesda, MD, USA) linked to a computer. An episcopic light through optical fibres allowed optimization of direction and intensity of light to obtain the most contrasted image possible.

For each numerical image, the software Shape 1.2 (Iwata and Ukai, 2002) calculated the Fourier coefficients in order

Table I. – Number of individuals collected at different areas for each species.

Species Sites	*Neogobius melanostomus*	*Ponticola eurycephalus*	*Mesogobius batrachocephalus*
Sulina	33	0	0
Constanta	63	0	0
23 August	39	2	25
Mangalia	62	23	45
Vama Veche	40	19	22
Total	237	44	92

to make them invariants to the otolith size and its orientation (and position) relative to the beginning of the outline, which is arbitrarily defined. The shape of each otolith was assessed with a Fourier series. Among several possibilities for analysing shape with Fourier series, the elliptic Fourier analysis (EFA) descriptors are often considered as more powerful than those issued from fast Fourier transformation (FFT) for shape analysis, and were therefore used in this work, although EFA is in fact less widely used than FFT (Tracey *et al.,* 2006; Gonzalez-Salas and Lenfant, 2007; Mérigot *et al.,* 2007). This technique describes the outline with several components, named harmonics. Each harmonic is characterized by four coefficients, resulting from the projection of each point of the outline on axes (x) and (y). The higher the number of harmonics, the greater is the accuracy of the outline description (Kuhl and Giardina, 1982). In addition, the Fourier power (FP) spectrum was calculated in order to determine the optimum number of harmonics necessary for the best reconstruction of the otolith outline (Crampton, 1995). The Fourier power of a harmonic is proportional to its amplitude and provides a measure of the amount of "shape information" described by this harmonic. For the nth harmonic, Fourier power (FP_n) is given by the expression:

$$FP_n = (A^2_n + B^2_n + C^2_n + D^2_n) / 2$$

where A_n, B_n, C_n and D_n are the Fourier coefficients of the nth harmonic.

Then, we can calculate the cumulated power percentage (FP_c) defined by:

$$FP_c = \sum_1^n FP_n$$

For this purpose, we randomly chose two sub-samples of 40 otoliths each (the first for right otoliths, the second for left otoliths) for both species, and the threshold of 99.99% of the mean cumulated Fourier power was chosen to define the optimum number of harmonics to be considered in the analyses. As the first 20 harmonics reached 99.99% of the cumulated power for both right and left otoliths, the Fourier analysis indicated that the otolith shape of the studied species could be summarized by these 20 harmonics, *i.e.* 80 Fourier coefficients. However, the coefficients derived from the 1st harmonic were not taken into account, because the outline reconstructed with these coefficients is a simple ellipse resulting in maximum Fourier power. These coefficients would then mask the information derived from the other harmonics (Crampton, 1995). So 19 harmonics, and thus 76 Fourier coefficients, were used to analyse the data.

Data analysis

All data analyses were performed with the SPSS statistical (version 13) and R software. Canonical discriminant analyses (CDA) were used to determine differences between species and sites. The CDA were performed with the Fourier coefficients (19 harmonics, thus 76 coefficients). The objective of the CDA is to investigate the integrity of predefined groups, *i.e.* individuals belonging to a given sample, such as species, geographical area, or size-classes in our case, through finding linear combinations of descriptors that maximize the Wilks lambda (λ) (Ramsay and Silveman, 2005). The Wilks λ allows assessment of the performance of the discriminant analyses. This statistic is the ratio between the intra-groups variance and the total variance, and provides an objective means of calculating the chance-corrected percentage of agreement between real and predicted group membership. The values of λ range from 0 to 1, the closer λ is to 0, the better the discriminating power of the CDA is. To validate the performance of the CDA, a Cohen's kappa statistical test was used. This statistical analysis provides an objective means of calculating the chance-corrected percentage of agreement between actual and predicted group membership; kappa values range from 0 to 1, with 0 indicating the CDA yields no improvement over chance, and 1 indicating perfect agreement (Titus *et al.,* 1984).

Several CDA were performed and allowed determination of the relative positions of the three species studied, *Neogobius melanostomus*, *Ponticola eurycephalus* and *Mesogobius batrachocephalus*. Then, populations of *N. melanostomus* were discriminated at local scale. The dissimilarity between groups was evaluated by the Euclidian distance (*d*) between the barycentre of each group.

Age estimation

Otolith samples were immersed in essential oil (chamomile) and observed under a stereomicroscope. Under reflected light, the alternation of opaque and hyaline zones formed one annulus. Age was estimated along the axis from the core to the rostrum. Each otolith was read by two readers separately (a third reader was added when differences in age were found).

Individual age was related to sex, total length and total wet weight for the three species. Mean values and standard deviations were estimated by age for total length and total wet weight. For each age, differences in total length and total wet weight were tested between males and females using nonparametric Kruskal-Wallis tests. Previously, normality of residuals and homoscedasticity were checked by Shapiro tests and Levene tests. The proportion of females in each species population was also estimated and expressed in percentage.

RESULTS

Inter- and intraspecific otolith shape analyses

The otolith Fourier coefficients were used to show the relative position of the three species of Gobiidae studied (Fig. 3). The CDA performed with these data allowed a clear separation of the three species ($p < 0.05$). The gener-

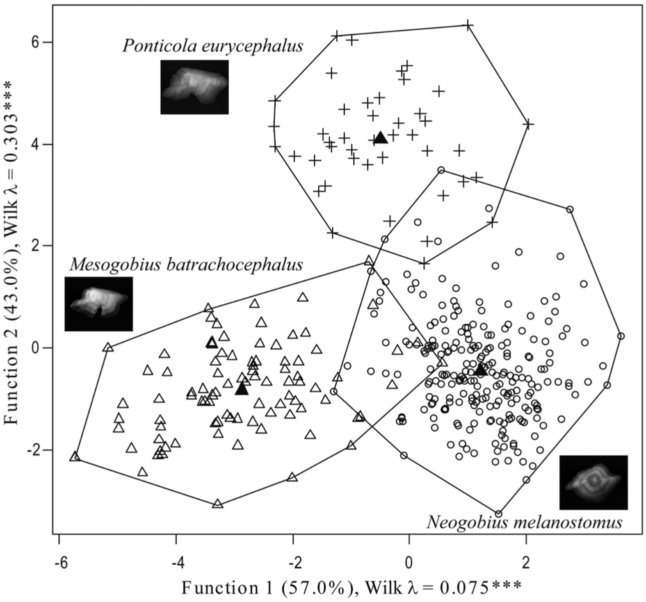

Figure 3. – Age structure of males and females for the three species.

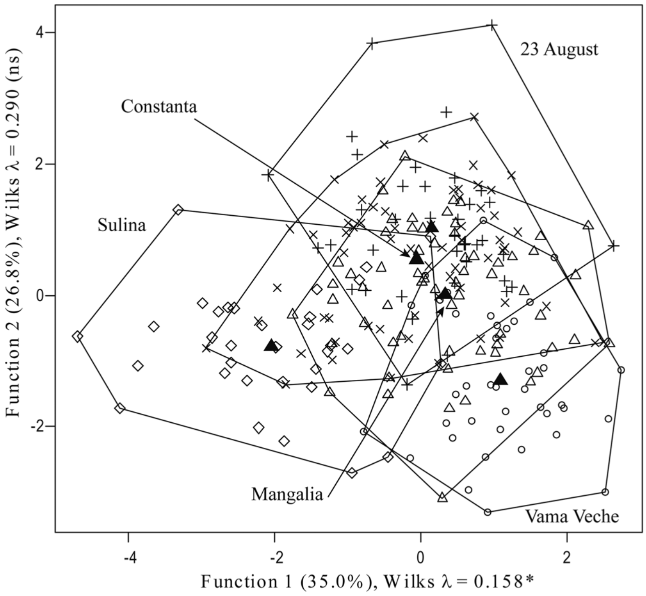

Figure 4. – Discrimination of the three species by otolith shape analysis.

Table II. – Euclidian distances between areas calculated from the Canonical Discriminant Analyses achieved for *Neogobius*.

d	Constanta	Mangalia	Sulina	Vama Veche
23 August	0.52	1.03	2.84	2.51
Constanta		0.67	2.39	2.18
Mangalia			2.50	1.51
Sulina				3.17

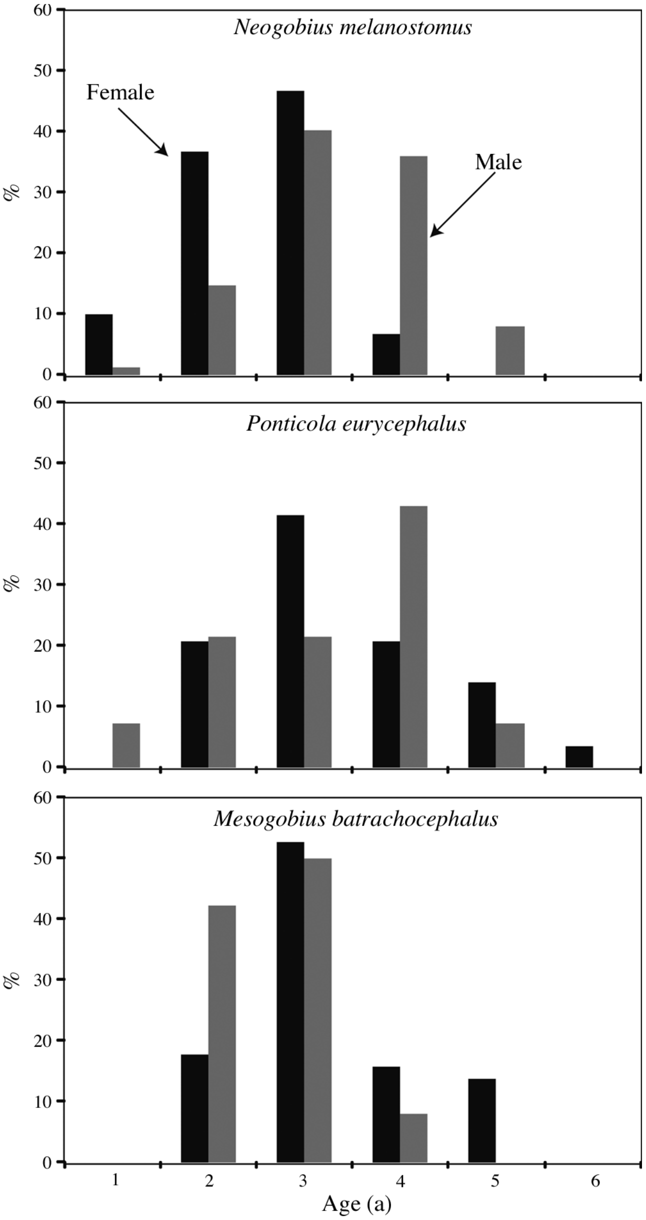

Figure 5. – Inter-area discrimination for *Neogobius melanostomus*.

al reclassification assessed with the Cohen-Kappa test was 92.4 ± 1.9%. *Neogobius melanostomus* (Nm) and *Mesogobius batrachocephalus* (Mb) were separate on the first discriminant function (d_{Nm-Mb} = 4.1), while the third species, *Ponticola eurycephalus (Pe)*, was separate on the second (d_{Nm-Pe} = 4.9 and d_{Pe-Mb} = 5.5).

CDA analysis was performed in order to search for inter-area comparison discriminations for *N. melanostomus*. The discrimination was significant with good reclassification (61.1 ± 3.8%). However (Fig. 4), only the first discriminant function was significant (Wilks λ1 = 0.158*) and revealed a clear discrimination of Sulina areas from the other areas

(Constanta, 23 Aug., Mangalia and Vama Veche) (Tab. II).

Age, sex and morphometrics of the studied species

Individuals aged three years represented the major proportion of fishes sampled: 42% of *N. melanostomus*, 35% of *P. eurycephalus* and 52% of *M. batrachocephalus*. The age structures of males and females of the three species did not show wide differences, except for males of *N. melanostomus*, which were older than the females ($p < 0.05$) (Fig. 5). Generally, no difference in length and mass was observed between males and females, except for males of *N. melanostomus* aged 3 and 4 years, and males of *P. eurycephalus* aged 2 years, that have greater length and mass than females of the same age ($p < 0.05$) (Tab. III). For both sexes, length and mass were higher at the same age for *M. batrachocephalus* than *P. eurycephalus,* and than *N. melanostomus*. The proportion of females in the population represented 27% for *N. melanostomus,* 67% for *P. eurycephalus* and 57% for *M. batrachocephalus*.

Table III. – Mean length and weight by species, age and sex. N= total number of analysed individuals; TL = total length; W = wet weight; SD = standard deviation; m = male; f = female; Diff. = difference.

Species	Age	Sex	N	TL (cm)		W (g)		Diff. in TL	Diff. in W
				Mean	± SD	Mean	± SD	$P < 0.05$	$P < 0.05$
N. melanostomus	1	m	2	7.6	1.1	6.6	3.4	ns	ns
		f	6	7.9	1.5	7.7	5.2		
	2	m	24	13.2	2.4	36.6	18.7	ns	ns
		f	22	12.4	2.1	30.4	15.4		
	3	m	66	15.3	2.2	53.7	23.7	m > f	m > f
		f	28	13.2	1.9	34.3	14.9		
	4	m	59	16.0	2.0	61.6	23.7	m > f	m > f
		f	4	12.6	1.1	32.0	8.1		
	5	m	13	17.9	2.1	87.6	28.1		
		f	–	–	–	–	–		
P. eurycephalus	1	m	1	13.0		17.8			
		f	–	–	–	–	–		
	2	m	3	14.9	1.7	51.8	15.3	m > f	m > f
		f	6	12.3	1.4	30.5	11.5		
	3	m	3	18.2	5.0	84.5	49.2	ns	ns
		f	12	14.4	1.9	52.5	20.3		
	4	m	6	15.5	2.4	62.4	22.2	ns	ns
		f	6	16.4	2.5	77.7	37.9		
	5	m	1	17.5		70.0		ns	ns
		f	4	16.7	1.6	71.9	18.9		
	6	m	–	–	–	–	–		
		f	1	17.8		74.4			
M. batrachocephalus	2	m	16	20.6	2.3	95.5	32.7	ns	ns
		f	9	19.5	2.3	77.5	30.7		
	3	m	19	22.6	2.3	137.3	47.2	ns	ns
		f	27	23.1	1.5	142.0	31.1		
	4	m	3	24.7	1.7	188.9	10.0	ns	ns
		f	8	27.1	3.5	233.8	115.6		
	5	m	–	–	–	–	–		
		f	7	30.0	1.2	338.4	65.0		

DISCUSSION

Otolith shape analyses

Gobies from the Ponto-Caspian region are members of an imperfectly defined group with remarkable historic endemism and taxonomic diversity. This group formed by ~24 species classified in four genera (*Apollonia, Mesogobius, Neogobius* and *Proterorhinus*) have been variously termed "neogobiids" (Neilson and Stepiens, 2009) and included in the "Gobius-lineage" (Agorreta *et al*., 2013). Their systematic relationships have been disputed and are unclear (Mihălcescu, 2005). This phylogenetic confusion is highlighted by the fact that many species from this group are invasive (including the round goby *N. melanostomus*) in freshwater systems of Eastern and Central Europe and North America (French and Jude, 2001; Vangerplorg *et al.,* 2002). As opposed to molecular phylogenies, variation in otolith shape is also a useful tool for discriminating species (L'Abée-Lund, 1988; Tuset *et al.,* 2006; Vignon and Morat, 2010). A recent paper used both phylogenetic methods and otolith shape analyses to distinguish between gobies of the genus *Ponticola* cryptic species (Vasilyeva *et al*., 2016).

Our results showed a clear differentiation of the three species (*N. melanostomus, P. eurycephalus* and *M. batrachocephalus*) occurring along the Romanian coast. This result is in agreement with recent molecular works on these three species (Neilson and Stepiens, 2009).

Discrimination of local teleost populations within species on the basis of otolith shape has been achieved in many areas (Atlantic Ocean, Pacific Ocean and Mediterranean) (Gonzalez-Salas and Lenfant, 2007; Mérigot *et al.,* 2007; Lord *et al.,* 2012; Morat *et al.,* 2012) but none in the Black Sea until now. These studies may however be useful to discriminate fish stocks, especially for these species, which are targeted by local fisheries. Several factors such as species behaviour (food or spatial-temporal niche differences) (Aguirre and Lombarte, 1999; Cardinale *et al.,* 2004; Mérigot *et al.,* 2007), differences in abiotic factors (temperature, salinity) (Gonzalez-Salas and Lenfant, 2007), or short-term variations in diet characteristics (Gagliano and McCormick, 2004) influence the otolith shape. The otolith shape of *N. melanostomus* collected in the North (Sulina) differed from those collected in the centre and the south (Constanta, 23 Aug., Mangalia and Vama Veche). Similar discrimination was observed for the Mullid subspecies *Mullus barbatus ponticus* (Morat *et al.,* 2012). These intra-specific spatial differences were probably due to both environment and feeding. The north-south distribution observed on the Romanian coast for *N. melanostomus* suggested a major role of environmental factors for this species. The northern area of the Black Sea is directly impacted by the Danube River inputs and presents lower salinity values (~4-5), higher turbidity, stronger currents and higher productivity than the southern area (Bănaru *et al.,* 2007). The variability of the abiotic parameters and of the productivity of coastal areas influenced by the Danube River inputs probably impact the abundance of *N. melanostomus* food resources (Bănaru and Harmelin-Vivien, 2009). Overall, all the three species studied showed a preference for bivalves. *N. melanostomus* consumes mainly bivalves, but also annelids and small teleosts (sprat) (Bănaru and Harmelin-Vivien, 2009). *P. eurycephalus* preyed on bivalves (Creţeanu, Pers. Comm.), while *M. batrachocephalus* consumes bivalves and small teleosts (gobies) (Creţeanu and Papadopol, 2006; Rosca and Mânzu, 2011). Spatial and seasonal variations were observed in their feeding behaviour (Bănaru and Harmelin-Vivien, 2009; Rosca and Mânzu, 2011). For example, *N. melanostomus* consumed more annelids in the northern area and more sprats in the south (Bănaru and Harmelin-Vivien, 2009). These differences in diet between areas may also explain spatial variations in otolith shape, as observed in our case and in other studies (Gagliano and McCormick, 2004; Morat *et al.,* 2012).

Age, sex and morphometrics of the studied species

Age and size represent important parameters to consider for local fishery management, as catches may influence species demography, size distribution and age-size ratio.

Individuals aged three years represented the major proportion of teleosts sampled and were sexually mature, suggesting a rather healthy demographic state. Generally, no difference in length and mass was observed between males and females, indicating that overall, they have the same environmental and growth conditions.

However, for the same species, individual mean size may also change with their trophic environment. The size of *N. melanostomus* was strongly variable according to the studied areas. In the Black Sea (present study) the size ranges were similar to those reported in the south-eastern Black Sea (Demirhan and Can, 2007) and intermediary between the highest reported values in Azov Sea (Simonovic *et al.,* 2001) and the lowest in the Baltic Sea (Sokolowska and Fey, 2011). These values could reflect the abundance of food resources related with the degree of eutrophication of these strongly productive marine environments, ranging from extreme eutrophic (Azov Sea) to eutrophic (Black Sea) and mesotrophic regimes (Baltic Sea) (de Leiva Moreno *et al.,* 2000). Differences observed in total length between these areas were probably related to the quantity of prey that influenced the growth of the fish. The size of *M. batrachocephalus* has been less extensively studied compared to *N. melanostomus*. However, the age-length distribution of *M. batrachocephalus* was similar to the pattern of distribution observed along the Romanian Coast in 2003-2005 (Creţeanu and Papadopol, 2006) and values were much higher than those reported in the south-eastern Black Sea (Demirhan and Can, 2007). These differences could reflect the higher productivity of plankton and more generally of abundant food resources in the Northwestern area of the Black Sea due to the large inputs of organic matter by the Danube River (Bănaru *et al.,* 2007).

The sex ratio is also important for the understanding of population dynamics and for fishery management. On the Romanian coast, *N. melanostomus* populations have a strongly imbalanced sex ratio in favour of males (2.7 times more), which may be a sign of unbalanced exploitation between females and males. This result was in accordance with populations studied in other areas: 1.9 times more males in the Baltic Sea (Kovtun, 1979 in Charlebois *et al.,* 1997) and 2.2 times more in the Trent-Severn River (Gutowsky and Fox, 2011). The sex ratio observed in *M. batrachocephalus* populations on the Romanian coast was imbalanced in favour of females (twice as many). This result was in agreement with observations of Creţeanu and Papadopol (2006) in the same area in 2005, but strongly differed from those made in 2003-2004 (1.5-3 times more in favour of males). Variations in sex ratio may be explained by differences between the sexes in length of reproduction, lifespan, migration, spatial distribution, and mortality rate during the reproductive season (Kvarnemo, 1994). Sex ratio in Gobiidae populations may impact the survival of fry. A predominance of females in a population leads to larger egg clutches that cannot be efficiently defended by males. The sex ratio can be impacted by fishing in spring due to the greater exposure of males (inshore), defending the spawning grounds.

P. eurycephalus showed a similar age-length distribution to *N. melanostomus* on the Romanian coast and the sex ratio was near equilibrium. However, the comparison with other areas was impossible due to the lack of studies on this species.

These results may be useful for the purposes of local fishery management and the analysis of goby populations in terms of size and age structure and relative to their spatial distribution. They may also provide a basis for the comparison of local populations from the Ponto-Caspian area with invasive populations from the Canadian lakes, the Baltic Sea or other locations.

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REFERENCES

AGORRETA A., SAN MAURO D., SCHLIEWEN U., VAN TASSELL J.L., KOVAČIĆ M., ZARDOYA R. & RÜBER L., 2013. - Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. *Mol. Phylogenet. Evol.,* 69: 619-633.

AGUIRRE H. & LOMBARTE A., 1999. - Ecomorphological comparisons of sagittae in *Mullus barbatus* and *M. surmuletus*. *J. Fish Biol.,* 55: 105-114.

BĂNARU D. & HARMELIN-VIVIEN M., 2009. - Feeding behavior of Black Sea bottom fishes: Did it change over time? *Acta Oecol.,* 35: 769-777.

BĂNARU D., HARMELIN-VIVIEN M., GOMOIU M.T. & ONCIU T.M., 2007. - Influence of the Danube River inputs on C and N stable isotope ratios of the Romanian coastal waters and sediment (Black Sea). *Mar. Poll. Bull.,* 54: 1385-1394.

BĂNARU D., HARMELIN-VIVIEN M. & BOUDOURESQUE C.F., 2010. - Man induced change in community control in the Northwestern Black Sea: the top-down bottom-up balance. *Mar. Environ. Res.,* 69: 262-275.

BĂNARU D., LE MANACH F., FÄRBER L., ZYLICH K. & PAULY D., 2015. - From bluefin tuna to gobies: a reconstruction of the fisheries catch statistics in Romania, 1950-2010. *In:* Atlas of Fisheries Impacts on the World's Marine Ecosystems, 1950-2010 (Pauly D. & Zeller D., eds), 10 p. Island Press, Washington, DC.

BERLINSKY N., BOGATOVA Y. & GARKAVAYA G., 2006. - Estuary of the Danube. *Handb. Environ. Chem.,* 5: 233-264.

CAMPANA S.E. & CASSELMAN J.L., 1993. - Stock discrimination using otolith shape analysis. *Can. J. Fish. Aquat. Sci.,* 50: 1062-1083.

CAMPANA S.E. & NEILSON J.D., 1985. - Mircrostructure of fish otoliths. *Can. J. Fish. Aquat. Sci.,* 42: 1014-1031.

CARDINALE M., DOERING-ARJES P., KASTOWSKY M. & MOSEGAARD H., 2004. - Effects of sex, stock and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Can. J. Fish. Aquat. Sci.,* 61: 158-167.

CHARLEBOIS P.M., MARSDEN J.E., GOETTEL R.G., WOLFE R.K., JUDE D.J. & RUDNIKA S., 1997. - The round goby, *Neogobius melanostomus* (Pallas), a review of European and North American literature. Illinois-Indiana Sea Grant Program and Illinois Natural History Survey. INHS Special Publication, 20: 1-76.

CRAMPTON J.S., 1995. - Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. *Lethaia,* 28: 179-186.

CREŢEANU M. & PAPADOPOL M.C., 2006. - Population structure, nutrition, reproduction of *Mesogobius batrachocephalus* (Pallas, 1811) (Pisces, Gobiidae) (Preliminary Data). *Cercet. Mar., INCDM, Constanţa*, 36: 319-340.

DE LEIVA MORENO J.I., AGOSTINI N.V., CADDY J.F. & CAROCCI F., 2000. - Is the pelagic-demersal ratio from fishery landings a useful proxy for nutrient availability? A preliminary data exploration for the semi-enclosed seas around Europe. *ICES J. Mar. Sci.*, 57: 1091-1102.

DEMIRHAN S.A. & CAN M.F., 2007. - Length-weight relationships for seven fish species from the southeastern Black Sea. *J. Appl. Ichthyol.*, 23: 282-283.

FAO, 2005. - Fishery Statistics. Capture production. Yearbook 100/1, 539 p.

FRENCH J.R.P. & JUDE D.J., 2001. - Diet and diet overlap of nonindigenous gobies and small benthic native fishes co-inhabiting the St. Clair River, Michigan. *J. Great Lakes Res.*, 27: 300-311.

GAGLIANO M. & McCORMICK M.I., 2004. - Feeding history influences otolith shape in tropical fish. *Mar. Ecol.-Prog. Ser.,* 278: 291-196.

GONZALEZ-SALAS C. & LENFANT P., 2007. - Interannual variability and intraannual stability of the otolith shape in European anchovy *Engraulis encrasicolus* (L.) in the Bay of Biscay. *J. Fish Biol.,* 70: 35-49.

GÜMÜŞ A. & KURT A., 2009. - Age structure and growth by otolith interpretation of *Neogobius melanostomus* (Gobiidae) from Southern Black Sea. *Cybium*, 33: 29-37.

GUTOWSKY L.F.G. & FOX G.F., 2011. - Occupation, body size and sex ratio of round goby (*Neogobius melanostomus*) in established and newly invaded areas of an Ontario River. *Hydrobiologia*, 671(1): 27-37.

IGLESIAS M., BROTHERS E. & MORALES-NIN B., 1997. - Validation of daily increment deposition in otoliths. Age and growth determination of *Aphia minuta* (Pisces: Gobiidae) from the northwest Mediterranean. *Mar. Biol.,* 129: 279-287.

IWATA H. & UKAI Y., 2002. - Shape: A computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *J. Hered.*, 93: 384-385.

KRITZER J.P., 2002. - Stock structure, mortality and growth of the decorated goby, *Istigobius decoratus* (Gobiidae), at Lizard Island, Great Barrier Reef. *Environ. Biol. Fish.,* 63: 211-216.

KUHL F.P. & GIARDINA C.R., 1982. - Elliptic Fourier features of a closed contour. *Comp. Graph. Image Process.*, 18: 236-258.

KVARNEMO C., 1994. - Temperature differentially affects male and female reproductive rates in the sand goby: consequences for operational sex ratio. *Proc. R. Soc. Lond.: Biol. Sci.,* 256: 151-156.

L'ABÉE-LUND J.H., 1988. - Otolith shape discriminates between juvenile Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*. *J. Fish Biol.,* 33: 899-903.

LECOMTE-FINIGER R., 1999. - L'otolithe : la "boîte noire" des Téléostéens. *Ann. Biol.*, 38: 107-122.

LORD C., MORAT F., LECOMTE-FINIGER R. & KEITH P., 2012. - Otolith shape analysis for three Sicyopterus (Teleostei: Gobioidei: Sicydiinae) species from New Caledonia and Vanuatu. *Environ. Biol. Fish.*, 93: 209-222.

MÉRIGOT B., LETOURNEUR Y. & LECOMTE-FINIGER R., 2007. - Characterization of local populations of the common sole *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. *Mar. Biol.,* 151: 997-1008.

MIHĂLCESCU A., 2005. - Systematic and ecological study of the gobiids (Pisces, Gobiidae) from Dobrudjan waters. PhD thesis, 218 p. Univ. Ovidius Constanţa. [in Romanian]

MORAT F., BETOULLE S., ROBERT M., THAILLY A.F., BIAGIANTI-RISBOURG S. & LECOMTE-FINIGER R., 2008. - What can otolith examination tell us about the level of perturbations of Salmonid fish from the Kerguelen Islands? *Ecol. Freshw. Fish.,* 17: 617-627.

MORAT F., LETOURNEUR Y., NÉRINI D., BĂNARU D. & BATJAKAS I.E., 2012. - Discrimination of red mullet populations (Teleostean, Mullidae) along multi-spatial and ontogenetic scales within the Mediterranean basin on the basis of otolith shape analysis. *Aquat. Living Res.,* 25: 27-39.

NEILSON M.E. & STEPIEN C.A., 2009. - Escape from the Ponto-Caspian: evolution and biogeography of an endemic goby species flock (Benthophilinae: Gobiidae: Teleostei). *Mol. Phylogenet. Evol.,* 52: 84-102.

NICOLAEV S. & ZAHARIA T., 2011. - Report on the marine and coastal environment state in 2011. Rapport INCDM Grigore Antipa, 65 p. [In Romanian]

PANFILI J., DE PONTUAL H., TROADEC H. & WRIGHT P.J. (Eds), 2002. - Manuel de Sclérochronologie des Poissons. 464 p. Coédition Ifremer-IRD, France.

PANIN N., 2005. - The Black Sea coastal zone – an overview. *Geo-Eco-Marina,* 11: 21-40.

PETRANU A., 1997. - Black Sea Biological Diversity – Romania. 354 p. Black Sea Environmental Series No. 4, United Nations Publications.

POSTOLACHE I., 1990. - The man's impact on the Romanian coastal zone. 33th CIESM Congress Proceedings, 32 p.

RAMSAY J.O. & SILVEMAN B.W., 2005. - Functional data analysis. 2nd edit., 310 p. New-York, USA: Springer.

ROSCA I. & MÂNZU C.C., 2011. - Feeding ecology of knout goby (*Mesogobius batrachocephalus* Pallas, 1814) from the Romanian Black Sea (Agigea – Eforie Nord area). *AACL Bioflux,* 4(2): 123-129.

SIMONOVIC P., PAUNOVIC M. & POPOVIC S., 2001. - Morphology, feeding, and reproduction of the round goby, *Neogobius melanostomus* (Pallas), in the Danube River basin, Yugoslavia. *J. Great Lakes Res.,* 27(3): 281-289.

SOKOLOWSKA E. & FEY D.P., 2011. - Age and growth of the round goby *Neogobius melanostomus* in the Gulf of Gdańsk several years after invasion. Is the Baltic Sea a new Promised Land? *J. Fish. Biol.,* 78(7): 1993-2009.

STEPIEN C.A. & TUMEO M.A., 2006. - Invasion genetics of Ponto-Caspian gobies in the Great Lakes: a 'cryptic' species, absence of founder effects, and comparative risk analysis. *Biol. Invasions,* 8: 61-78.

TITUS K., MOSHER J.A. & WILLIAMS B.K., 1984. - Chance-corrected classification for use in discriminant analysis: ecological applications. *Am. Midl. Nat.,* 111: 1-7.

TRACEY S.R., LYLE J.M. & DUHAMEL G., 2006. - Application of elliptical Fourier analysis of otolith form as a tool for stock identification. *Fish. Res.,* 77: 138-147.

THACKER C.E. & ROJE D.M., 2011. - Phylogeny of Gobiidae and identification of gobiid lineages. *Syst. Biodivers.,* 9: 329-347.

TUSET V.M., ROSIN P.L. & LOMBARTE A., 2006. - Sagittal otolith shape used in the identification of fishes of the genus *Serranus. Fish. Res.,* 81: 316-325.

VANGERPLORG H.A., NALEPA T.F., JUDE D.J., MILLS E.L., HOLECK K.T., LIEBIG J.R., GRIGOROVICH I.A. & OJAVEER H., 2002. - Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. *Can. J. Fish. Aquat. Sci.,* 59: 1209-1228.

VASILYEVA E.D., SCHWARZHANS W.W., MEDVEDEV D.A. & VASILYEV V.P., 2016. - Cryptic species of ponto-caspian bighead goby of the genus *Ponticola* (Gobiidae). *J. Ichthyol.,* 56: 1-18.

VIGNON M. & MORAT F., 2010. - Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. *Mar. Ecol.-Prog. Ser.,* 411: 231-241.

XIN Y., LIANG C., JINHU L., BO Z., XIUJUAN S. & SHUOZENG D., 2014. - Application of otolith shape analysis for stock discrimination and species identification of five goby species (Perciformes: Gobiidae) in the northern Chinese coastal waters. *Chin. J. Oceanol. Limnol.,* 32: 1060-1073.